PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Paul F. Umbeck
Serial No.: 937,384
Filed: December 3, 1986
For: GENETIC ENGINEERING OF COTTON PLANTS AND LINES

Date: October 26, 1989
Group No.: 184
Examiner: D. Fox
Docket No.: D601CETUS40
File No.: 11-229-9025-3

Commissioner of Patents
and Trademarks
Washington, D.C. 20231

APPELLANT'S BRIEF

The appellant of the above-identified patent application filed on May 26, 1989 a Notice of Appeal From the Primary Examiner to the Board of Appeals, appealing the decision of the Primary Examiner mailed January 3, 1989, finally rejecting Claims 1 - 23 in the above-identified patent application. This Brief is in support of said appeal.

STATUS OF THE CLAIMS

The status of the claims of this application is that the presently pending claims are Claims 2 - 23. While Claims 1 - 23 were examined in the Office Action of January 3, 1989, Claim 1 was cancelled by the applicant's Amendment dated May 2, 1989, as entered by the Examiner in an Advisory Action dated May 22, 1989.

STATUS OF AMENDMENT AFTER FINAL

It is the understanding of the applicant, as communicated to the undersigned by Examiner Fox, that upon entry of the appeal in this matter, the applicant's Amendment filed May 2, 1989 would be entered into the application. A statement to this effect appears in the Advisory Action dated May 22, 1989. Accordingly, it is the applicant's understanding and belief that the Amendment filed by the applicant May 2, 1989 has been entered into the file of the application.

SUMMARY OF THE INVENTION

The present invention is directed to the genetic transformation of plants and lines of cotton. Such genetic transformation, also referred to as genetic engineering, involves the insertion of a foreign gene into the genome, or inherited nuclear genetic material, of a cotton plant in such a fashion that the foreign gene can be inherited by the progeny of the plant. Through such genetic transformation, the insertion of desired traits into cotton plants and their progeny, referred to as lines, can be accomplished through the techniques of recombinant genetic technology.

The applicant here has achieved the genetic engineering of cotton through the use of the plant genetic engineering vector Agrobacterium tumefaciens, a soil-dwelling microorganism which

has the ability to transfer a portion of its plasmid DNA, referred to as T-DNA, into the genome of infected plant cells.

The applicant here has developed a method for introducing genes into the cotton plants, which method involves exposing a certain tissue type of immature cotton plants, known as the hypocotyl, to a culture of Agrobacterium tumefaciens harboring a T-DNA including the foreign gene desired to be entered into the cotton plants. The exposed cotton hypocotyl tissue is then cultured in the presence of selection agents, which select for a resistance gene also incorporated into the DNA included in the Agrobacterium tumefaciens T-DNA plasmid. The cotton tissue thus exposed to a selection agent is then induced to form somatic embryos, which is a type of cotton tissue which can be regenerated into whole cotton plants. The cotton plants thus genetically transformed by the applicant proved to be sexually fertile, and were capable of passing on the introduced foreign genes to their progeny.

The applicant's invention is both this method of genetically engineering cotton, involving hypocotyl based system of Agrobacterium-mediated transformation, and also the novel plants thus produced. The applicant's disclosure is believed to describe the first genetically transformed cotton plants ever achieved or reported.

CONCISE STATEMENT OF ALL ISSUES

It is believed that there is only one unresolved issue regarding the claims on appeal in this patent application. That issue is a single rejection applied by the Examiner, under 35 U.S.C. Section 103, against all the claims of the applicant. The Examiner asserts that all the claims of the applicant are obvious to one of ordinary skill in the art over references to DeBlock et al., taken in conjunction with references to Zutra and Rangan et al., as applied by the Examiner in the Office Action of February 3, 1988, and further explained in the Office Action of January 3, 1989.

GROUPING OF CLAIMS

The applicant believes the claims of the present patent application are properly grouped as follows:

a. A method of introducing genes into cotton plants and lines as recited in Claims 2 - 13 and 22;

b. Cotton plants claimed as a product-by-process of the above method claims, recited in Claims 14 - 16; and

c. Cotton plants and seed as an independent product, as recited in Claims 17 - 21 and 23.

It is believed by the applicant that the three groups of claims are separately patentable and appropriate arguments to the same will be presented below.

ARGUMENT

Rejection of Method Claims.

The Examiner has rejected all of the claims pending in the application in a single rejection under 35 U.S.C. Section 103, based on a combination of three prior art references. Accordingly, a brief review of the three references is essential to an understanding of the merits of the rejection, and the applicant's arguments as to the distinction between his method and the combination as cited by the Examiner.

The principal reference applied by the Examiner is that to DeBlock et al. DeBlock is a 1984 publication which teaches the expression of foreign genes in regenerated tobacco plants. While text of the DeBlock article often refers to "plants" rather generically, it does not explain which plants are susceptible to the method disclosed in the publication, and which are not. DeBlock does illustrate and describe the genetic engineering of tobacco utilizing a transformation technique making use of the Ti plasmid of Agrobacterium tumefaciens, and describes the use of selectible markers introduced into the tobacco cells through the Agrobacterium-mediated transformation technique, which markers include resistance traits to kanamycin and chloramphenicol. The method used by DeBlock involves the co-culturing of naked tobacco cells, referred to as protoplasts, with the Agrobacterium, and then the culture of the resulting tobacco cells into an undifferentiated mass, referred to as a callus. Cells in the tobacco callus were then subjected to a selective media so that

non-transformed cells were killed, resulting in a callus which is wholly or at least mostly transformed. Tobacco shoots were then induced to form in the callus which were regenerated into whole tobacco plants.

The first secondary reference applied by the Examiner is a reference to Zutra. Zutra is a 1982 paper which observes that a species of Agrobacterium, known as Agrobacterium radiobacter, is capable of infecting the roots of cotton plants in cotton fields. This reference is cited by the Examiner to demonstrate that field cotton is a host for Agrobacterium infection. Applicant admits that cotton is a natural host for infection by oncogenic Agrobacterium strains.

The Rangan et al. paper cited by the Examiner is an abstract from a presentation at a 1984 meeting which describes a tissue culture technique found useful in cotton. This paper, which deals totally with non-transformed cotton tissues, describes how callus tissues may be established from somatic cotton tissues when properly introduced into tissue culture. Such callus cultures of cotton could be induced to form somatic embryos, which are differentiated tissues giving rise to embryonic plants and which are distinguished from naturally occurring plant embryos because of their origin, i.e. they are induced to form from culture of somatic cells. The Rangan paper reported the regeneration of cotton plantlets from such somatic embryos.

To further understand why the rejection as created by the Examiner is improperly applied to the method claims of the present application, it is next appropriate to examine the

claimed subject matter to examine how that claimed subject matter differs from the references cited by the Examiner. Referring to Claim 2, the first step consists of exposing hypocotyl tissue of immature cotton plants to a culture of transformation competent non-oncogenic Agrobacterium tumefaciens harboring a Ti plasmid in which the T-DNA region includes both a foreign chimeric gene and a selection agent resistant gene, both genes constructed so as to be expressible in the cells of cotton plants. Notice that there are several limitations in this claimed step. It is hypocotyl tissue, in particular, of immature cotton plants that is exposed, not simply any tissue from the cotton plants. Note also that the Ti plasmid used as the transfer agent to transfer the DNA into the transformed cotton tissue includes a dual set of genes, a foreign chimeric gene of interest, and a selection agent resistance gene.

The next step in the method of Claim 2 is to culture the exposed tissue in the presence of the selection agent for which the resistance gene encodes, so as to select for cotton cells which are transformed with the T-DNA region from the Agrobacterium tumefaciens.

The following step is the induction of somatic embryo formation in the exposed tissue in culture. The final step is regenerating those somatic embryos into whole cotton plants.

First, it is worthy of note that certain of the express limitations of the method of Claim 2 are not found in any of the references. No reference teaches, or suggests, exposing hypocotyl tissue of cotton to Agrobacterium to obtain transformed

tissues. In fact, no teaching of transforming hypocotyl tissue of any plant is found in any of the cited references. In addition, no reference suggests that antibiotic resistance markers, such as those used by the applicant, are effective in cotton tissues. The DeBlock reference teaches that certain selectible resistance markers, including NPT II (neomycin phosphotransferase) and CAT (chloramphenicol acetyltransferase), are effective in tobacco. But where is the evidence, or suggestion, that these same selection agents would work effectively if expressed in cotton cells? There is none. Apparently the Examiner argues that if these agents work in tobacco, they should work in all plants. There is no evidence to support this assertion. In fact, enclosed herewith is a recent report on transforming another important crop species, soybean, which reports that these same selection agents are so poorly expressed in soybean so as to be impractical selection agents for conventional Agrobacterium transformation. Tibtech, 6 pp. 265-266 (Nov 1988) (copy enclosed). If the selection agents work in some species and not others, why is it obvious that they would work in cotton?

What the Examiner has done in the rejection is to parse the applicant's method steps and separately seek references which individually show each of the steps separately from the others. Having found some of the individual parts of the applicant's overall method in a variety of references, the Examiner seeks then to apply the logic of hindsight to find that the applicant's

combination of those steps is obvious. It is believed that the logic of this rejection is inappropriate.

Several facts about the background and the state of the art at the time of the applicant's invention are enormously instructive to the comprehension of the present invention. First, there is no known report, and certainly no cited report, of anyone ever successfully genetically engineering a cotton plant, prior to the work of the applicant here. In other words, the achievement of the applicant in creating a genetically transformed cotton plant, by whatever method, is entirely unprecedented. It should be a matter about which this body could take judicial notice that cotton is a very important crop in the United States and worldwide. Yet, prior to the efforts of the applicant here, it was not genetically transformed, or transformable.

At the time of the filing of the present patent application, as the record shows, the technology of plant genetic engineering was at a stage in which only the genetic engineering of several model species could be accomplished with any predictability and regularity. These model species include tobacco, petunia, and carrot. In addition, the genetic engineering of tomato, turnip, and potato had been reported. Note that the applicant's publication of his own research results, cited in the file of this application, correctly report that these model plants have all been genetically engineered. Nevertheless, commercially important field crop plants had not proven susceptible to genetic engineering as of that time. These field crop plants included

corn, soybean, wheat, rice, and, at least until the work of the applicant was published, cotton.

It was true that it was a hope of many in the field of plant genetic engineering that most plants would ultimately be genetically engineered. However, the exact mechanism by which each individual crop plant could be genetically engineered was not yet developed.

The Examiner apparently believes that because Agrobacterium-mediated transformation had been effective for certain plant species, it was therefore obvious to apply an Agrobacterium-mediated transformation technique to any plant which was a susceptible host for Agrobacterium infection. The applicant respectfully asserts that there is no indication on the record, or in the references, that plant genetic engineering is that easy. In order to obtain a wholly transformed plant, as carefully recited in the specification, two processes are necessary. One process is the genetic transformation of a particular tissue or cell type. The next process is the regeneration of a whole plant from that tissue or cell type in culture. The two halves of the process must be matched, that is a reproducible mechanism must exist for transforming the cell type and the cell type must be one which is readily regenerable. Not all tissues or cell types are susceptible to the same transformation methodologies and for many plants, such as cotton, only certain cell types or structures can be regenerated into whole plants. The Examiner seeks to use the superior vantage point of hindsight to suggest from certain totally unrelated and

disconnected references that the combination of method steps of the applicant, as recited in Claim 2, are obvious. It is asserted by the applicant that the sum of the cited prior art does not suggest either the particular method employed by the applicant or any reasonable likelihood that the particular method employed by the applicant would ultimately be successful in genetically engineering cotton plants.

As an illustration of the lack of teachings which would suggest the efficacy of the applicant's process, note again that the process as recited by the applicant relies on the selection of transformed tissues through the use of a selection agent. The specification in the claims recite several antibiotic agents which have been found to be useful selection agents in cotton such as kanamycin and chloramphenicol. The Examiner gives little or no patentable weight to this selection presumably because the reference to DeBlock shows selection of transformed tissues using those same two antibiotics. Only the reference to DeBlock teaches that the antibiotics are successful selectible agents in tobacco. Nowhere is there a teaching, or a suggestion, of a sufficient fundamental understanding of the biochemistry of cotton plants sufficient to predict in advance that the same antibiotic agents would be successful selectible markers in cotton. In other words, what the references suggest is an area of logical inquiry, they do not suggest or teach that the area of inquiry could be suggested to be successful with any degree of certainty.

The applicant understands that the CAFC has held that "obviousness does not require absolute predictability of success." In re O'Farrell, 853 F.2d 894, 903, 7 U.S.P.Q. 2d 1673 (Fed. Cir. 1988). However, in O'Farrell, the prior art "contained detailed enabling methodology for practicing the claimed invention, a suggestion to modify the prior art to practice the claimed invention, and evidence suggesting that it would be successful." O'Farrell, 835 F.2d at 902. The rejection here fails all three parts of this test. The references cited here do not contain a detailed enabling methodology for practicing the present invention, since none of the references actually applied by the Examiner either suggests or discusses the transformation of hypocotyl tissues of cotton. Secondly, the Examiner has cited no reference which suggests that one may successfully apply an Agrobacterium-mediated transformation technique to cotton. Thirdly, there is also no teaching anywhere which suggests that an Agrobacterium-mediated transformation of cotton would be successful, or that an antibiotic selection medium in this specific plant would work. Thus, there is certainly no substantial evidence indicating that the application of all these techniques, in combination, would be successful in creating genetically engineered cotton plants, as the applicant has succeeded in doing. Accordingly, there is neither a detailed methodology, a suggestion to modify the prior art, or evidence suggesting it would be successful.

The fact situation of this rejection is one of those situations in which an Examiner has found the various parameters

of an applicant's claim in various different references, but has found no reference indicating how to, or the desirability of, combining the different teachings. The situation is analogous to that of In re Geiger, 815 F.2d 686, 2 U.S.P.Q. 2d 1276 (Fed. Cir. 1987) where the applicant claimed a method of inhibiting scale formation on metal parts by use of a composition containing three components. The application was rejected as obvious under Section 103 on the grounds that each of the three components had been conventionally used in the art of treating cooling water systems. However, the Federal Circuit reversed the Patent and Trademark Office finding that "at best, in view of these disclosures, one skilled in the art might find it obvious to try various combination of these known agents. However, this is not the standard of 35 U.S.C. § 103." Geiger, 815 F.2d at 688. Here the facts are very analogous. What the Examiner has suggested is that some of the method steps as used by the applicant here have been used in other crop plants to create genetically engineered plants. This suggests that one in the art might want to try one or another combination of those techniques on cotton. However, as it has become trite to repeat, the standard under Section 103 is not "obvious to try."

It should not go unnoted that this case involves the essential molecular biology of plants. The molecular biology of plants, such as cotton, is not well understood even by those of greater than ordinary skill in the art. Here, given the fact that cotton could be a host for Agrobacterium infection, and given the fact that Agrobacterium had proven a successful medium

for the genetic engineering of other Agrobacterium-susceptible plants, there was arguably reason to try Agrobacterium as a vector for genetically engineering cotton. These facts alone, however, do not make it obvious that such a procedure could actually be accomplished. In addition, the step of selectible resistance had yet to be demonstrated, and its functionality in cotton could not be predicted, with even a modest degree of confidence. Therefore, this combination of references, even if taken as a whole, merely suggest an avenue of fruitful inquiry. They do not suggest that the genetic engineering of cotton plants, an unprecedented achievement, could actually prove to be practical.

In addition, the combination of references suggested by the Examiner does not give the method of the present invention. DeBlock shows transforming plant cells by co-cultivating Agrobacterium with plant protoplast cultures. Rangan shows the regeneration of cotton plants from somatic embryos. But there is no teaching on how to culture cotton protoplasts into somatic embryos. No such teaching is cited, because no present technology existed at the time of the present invention to accomplish such a technique. Certainly, neither reference suggests the hypocotyl cultivation method of the present invention. So the references, even if they were all combined, still do not teach the method of the present invention.

Finally, it must be noted that the Examiner's rejection relies on irrelevant facts. The applicant argued in prior responses that the combined references failed to suggest the

hypocotyl inoculation techniques for cotton used by the applicant here. The Examiner responded, in the Office Action of January 3, 1989 that the applicant's use of hypocotyl transformation was not a persuasive distinction over the prior art since other tissue types, such as cotyledon explants, as demonstrated by Firoozabady et. al. could also be used. This logic is defective for two reasons. First, the standard of patentability is not optimization, it is obviousness. Applicant did not argue that the hypocotyl method was the best, but simply that it was not obvious based on the cited references. The fact that other tissues also may be used does not make the use of this tissue type obvious. Secondly, the publication by Firoozabady was well after the publication of the applicant's work, and well after the filing date of this patent application. That paper is under no circumstance prior art, and teaches nothing about the prior art at the time the invention was made. Note that the Firoozabady paper cites the work of the applicant several times. Therefore this argument, insofar as it is part of the grounds of rejection, is entirely improper.

It is therefore believed that the method as described and claimed in the patent application of the applicant here is not obvious in view of any of the cited prior art references, and is not in any way suggested by their combination.

REJECTION OF PRODUCT-BY-PROCESS CLAIMS

Claims 14 - 16 present product-by-process claims to cotton plants produced by the method of Claim 2. Although the same combination of references were applied against these claims as against the method claims, the rejection is even weaker for these claims.

These claims, of course, are directed to genetically engineered cotton plants made by the method discussed above. A review of the references reveals no teaching of genetically engineered cotton, regardless of method made. In fact, there is not even a suggestion in any of the cited references of either the possibility or the desirability of genetically engineered cotton plants.

Again, the Examiner has applied the same combination of references against these claims. Also again, there is no suggestion how these references may be combined to create genetically engineered cotton plants. The transformation technique of DeBlock, while useful in tobacco, is inapplicable to cotton plants. Since the method of making the claimed plants is not obvious, and since none of the references either suggests or discloses genetically engineered cotton plants made by this method, the product-by-process Claims 14 - 16 are also properly allowable over the art of record.

REJECTION OF PRODUCT CLAIMS

Claims 17 - 21 and 23 are directed to cotton plants which have inserted into the genome, or their inheritable genetic material, a foreign gene construction effective to express a foreign cellular product in the cells of the cotton plants. These claims were also subjected by the Examiner to the same obviousness rejection, with the same combination of references.

The limitations of these claims not found in the prior art should be clear based on the prior discussion. No prior art reference teaches genetically engineered cotton plants or seeds. These product claims recite that a detectable cellular product, coded for by the inserted foreign gene, is found in the claimed plants. This limitation is neither found in the references nor suggested by them.

The applicant fails to find even a single mention or suggestion in the cited references of even the possibility of a genetically engineered cotton plant. To combine the references as suggested by the Examiner is to reverse engineering the invention working from the applicant's achievement. This is not the proper basis for rejection.

The rejection of the product claims is improper for all the reasons discussed in conjunction with the method claims above. The Examiner's rejection is a variant of the impermissible "obvious to try" rejection. There is no suggestion to combine these references in the way suggested by the Examiner. There is

no reasonable basis to believe that such a combination would work, even if the combination were suggested.

However, there is even a more basic flaw in the rejection of these product claims as obvious. None of the prior art teachings, or their combination, enables the creation of genetically transformed whole cotton plants. DeBlock might arguably suggest that cotton protoplasts in culture could be transformed, but no reference teaches how to regenerate a plant from such a cotton protoplast. Rangan teaches regeneration of cotton from an embryogenic cotton callus culture, but no reference teaches how to transform such an embryonic cotton culture. Thus, there is not even a logical chain in the combined references on how to create a transformed whole cotton plant. How can such a claimed product be obvious when there is not even a sufficient teaching in the prior art that would enable one to make the product?

The Examiner seeks to bridge this logical gap by mere arguments of obviousness. As stated in the Office Action of January 3, 1989, "The Examiner maintains that choice of explant for transformation would be the optimization of process parameters, given the teaching of cotton root and cotyledon inoculation taught by Zutra et. al. and Firoozabady et. al. respectively." It is a logical impossibility to argue that a product can be created by merely optimizing process parameters, where the prior art processes do not enable the creation of the product. Zutra shows that Agrobacterium can infect cotton roots, but no reference is cited to show cotton regeneration from roots.

Firoozabady et. al. is not a valid reference, since Firoozabady's teaching is after that of the applicant here, not before. Therefore this assertion by the Examiner is without proper legal, or scientific, basis.

The applicant here was the first to genetically engineer cotton and the first to enable others to do likewise. The applicant demonstrated, for the first time, that Agrobacterium-mediated transformation techniques could be applied to at least one cotton tissue, that selectible antibiotic markers could be effectively used to select transformed cotton cells, and that cotton tissues which could be transformed could be regenerated. The success of all these parts of the applicant's achievement could not have been predicted with reasonable certainty in advance.

Therefore, since the applicant's cotton plants and seeds claimed in Claims 17 - 21 and 23 contain limitations not found in the prior art references, and since the prior art references would not even enable the creation of these plants or seeds, these product claims cannot rightfully be considered as obvious.

CONCLUSION

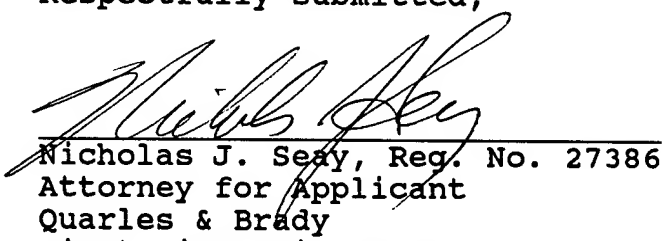
In view of the foregoing, a reversal of the Final Rejection of Claims 2 - 23 in the Office Action dated January 3, 1989 is respectfully requested.

A separate petition under 37 CFR §1.136 is submitted

herewith so that this Brief will be considered as timely filed.

A separate appendix reprinting Claims 2 - 33 follows.

Respectfully submitted,



Nicholas J. Seay, Reg. No. 27386
Attorney for Applicant
Quarles & Brady
First Wisconsin Plaza
P. O. Box 2113
Madison, WI 53701-2113

(608) 251-5000

NJS10/03

2. A method of introducing genes into cotton plants and plant lines comprising the steps of:

exposing hypocotyl tissue of immature cotton plants to a culture of transformation competent non-oncogenic Agrobacterium tumefaciens harboring a Ti plasmid having a T-DNA region including both a foreign chimeric gene and a selection agent resistance gene, both genes including appropriate regulatory sequences so as to be expressed in the cells of cotton plants;

culturing the exposed tissue in the presence of a selection agent for which the resistance gene encodes for resistance so as to select for plant cells transformed with the T-DNA region;

inducing somatic embryo formation in the exposed tissue in culture; and

regenerating the somatic embryos into whole cotton plants.

3. The method of Claim 2 wherein said exposing step is preceded by surface sterilization of cotton seeds followed by germination of said cotton seeds to form said immature cotton plants.

4. The method of Claim 2 wherein the hypocotyl tissue comprises pieces of hypocotyl explants which are removed from said immature cotton plants.

5. The method of Claim 2 wherein the culture of Agrobacterium tumefaciens harbors a binary Ti plasmid system in

which a virulence trait is carried on a plasmid separate from the plasmid carrying the T-DNA region.

6. The method of Claim 5 wherein the T-DNA region includes only the T-DNA right and left borders from the T-DNA of a wild-type Ti plasmid.

7. The method of Claim 2 wherein the selection agent is an antibiotic and the resistance gene codes for antibiotic resistance.

8. The method of Claim 7 wherein the antibiotic resistance gene is the NPTII gene and the antibiotic is selected from the group consisting of Kanamycin and G418.

9. The method of Claim 7 wherein the antibiotic resistance gene is the CAT gene and the antibiotic is Chloramphenicol.

10. The method of Claim 7 wherein two antibiotics and two antibiotic resistance genes are used.

11. The method of Claim 10 wherein in the two antibiotics, one is selected from the group consisting of Hygromycin B and Chloramphenicol, and one is selected from the group consisting of Kanamycin and G418.

12. The method of Claim 2 further including, after the step of exposing the tissue to said Agrobacterium tumefaciens, culturing the tissue on a medium containing at least one antibiotic toxic to said Agrobacterium tumefaciens but not toxic to cotton cells.

13. The method of Claim 2 wherein the step of inducing embryo formation includes culturing the plant tissue on a culture media containing at least one auxin or cytokinin.

14. Cotton plants produced by the method of Claim 2 which include cells which comprise in their genome the foreign chimeric recombinant gene and the selection agent gene and which produce a foreign cellular product coded by the foreign gene.

15. Cotton somatic embryos produced by the method of Claim 2.

16. Cotton seeds produced by the plants of Claim 14.

17. Cotton seed capable of germination into cotton plants comprising in their genome a chimeric recombinant gene construction including a foreign gene and promoter and control sequences operable in cotton cells, the chimeric gene construction being effective in the cells of the cotton plant to express a cellular product coded by the foreign gene, the cellular product imbuing the plant with a detectable trait

selected from the group consisting of a foreign protein and a negative strand RNA.

18. Cotton plants germinated from the seeds of Claim 17.

19. Cotton seeds as claimed in Claim 17 wherein the cellular product is selected from the group consisting of an exogenous protein and an RNA selected to produce a somatic change to the cotton plant.

20. Cotton seeds as claimed in Claim 17 wherein the foreign gene codes for the production of a negative RNA strand effective to condition a somatic change in the cotton plant grown from the seed.

21. Cotton seeds as claimed in Claim 19 wherein the promoter sequence is selected from the group consisting of the nopaline synthase promoter from Agrobacterium tumefaciens and the cauliflower mosaic virus 35s promoter.

22. A method for introducing genes into cotton plants and plant lines, comprising the following steps in sequence:

a) surface sterilizing cotton seeds;

b) allowing said cotton seeds to germinate thus forming immature cotton plants, said immature cotton plants including hypocotyl tissue;

c) exposing said hypocotyl tissue to a culture of transformation competent non-oncogenic Agrobacterium tumefaciens harboring a Ti plasmid having a T-DNA region including both a foreign chimeric gene and a selection agent resistance gene;

d) culturing said hypocotyl tissue on a medium containing at least one antibiotic toxic to said Agrobacterium tumefaciens but not toxic to cotton cells;

e) culturing said tissue of step d) in the presence of a selection agent for which the resistance gene encodes for resistance so as to select for plant cells transformed with the T-DNA region;

f) inducing somatic embryo formation in the exposed tissue in culture; and

g) regenerating the somatic embryos into whole cotton plants.

23. A cotton plant comprising in the genome of at least some of its cells a foreign gene construction including promoter and control sequences effective in cotton cells and a heterologous coding sequence, the foreign gene construction effective to cause expression of a detectable cellular product coded by the heterologous coding sequence in the plant cells, the cellular product selected from the group consisting of a foreign protein and a negative strand RNA.

NJS6/08

2. A method of introducing genes into cotton plants and plant lines comprising the steps of:

exposing hypocotyl tissue of immature cotton plants to a culture of transformation competent non-oncogenic Agrobacterium tumefaciens harboring a Ti plasmid having a T-DNA region including both a foreign chimeric gene and a selection agent resistance gene, both genes including appropriate regulatory sequences so as to be expressed in the cells of cotton plants;

culturing the exposed tissue in the presence of a selection agent for which the resistance gene encodes for resistance so as to select for plant cells transformed with the T-DNA region;

inducing somatic embryo formation in the exposed tissue in culture; and

regenerating the somatic embryos into whole cotton plants.

3. The method of Claim 2 wherein said exposing step is preceded by surface sterilization of cotton seeds followed by germination of said cotton seeds to form said immature cotton plants.

4. The method of Claim 2 wherein the hypocotyl tissue comprises pieces of hypocotyl explants which are removed from said immature cotton plants.

5. The method of Claim 2 wherein the culture of Agrobacterium tumefaciens harbors a binary Ti plasmid system in

which a virulence trait is carried on a plasmid separate from the plasmid carrying the T-DNA region.

6. The method of Claim 5 wherein the T-DNA region includes only the T-DNA right and left borders from the T-DNA of a wild-type Ti plasmid.

7. The method of Claim 2 wherein the selection agent is an antibiotic and the resistance gene codes for antibiotic resistance.

8. The method of Claim 7 wherein the antibiotic resistance gene is the NPTII gene and the antibiotic is selected from the group consisting of Kanamycin and G418.

9. The method of Claim 7 wherein the antibiotic resistance gene is the CAT gene and the antibiotic is Chloramphenicol.

10. The method of Claim 7 wherein two antibiotics and two antibiotic resistance genes are used.

11. The method of Claim 10 wherein in the two antibiotics, one is selected from the group consisting of Hygromycin B and Chloramphenicol, and one is selected from the group consisting of Kanamycin and G418.

12. The method of Claim 2 further including, after the step of exposing the tissue to said Agrobacterium tumefaciens, culturing the tissue on a medium containing at least one antibiotic toxic to said Agrobacterium tumefaciens but not toxic to cotton cells.

13. The method of Claim 2 wherein the step of inducing embryo formation includes culturing the plant tissue on a culture media containing at least one auxin or cytokinin.

14. Cotton plants produced by the method of Claim 2 which include cells which comprise in their genome the foreign chimeric recombinant gene and the selection agent gene and which produce a foreign cellular product coded by the foreign gene.

15. Cotton somatic embryos produced by the method of Claim 2.

16. Cotton seeds produced by the plants of Claim 14.

17. Cotton seed capable of germination into cotton plants comprising in their genome a chimeric recombinant gene construction including a foreign gene and promoter and control sequences operable in cotton cells, the chimeric gene construction being effective in the cells of the cotton plant to express a cellular product coded by the foreign gene, the cellular product imbuing the plant with a detectable trait

selected from the group consisting of a foreign protein and a negative strand RNA.

18. Cotton plants germinated from the seeds of Claim 17.

19. Cotton seeds as claimed in Claim 17 wherein the cellular product is selected from the group consisting of an exogenous protein and an RNA selected to produce a somatic change to the cotton plant.

20. Cotton seeds as claimed in Claim 17 wherein the foreign gene codes for the production of a negative RNA strand effective to condition a somatic change in the cotton plant grown from the seed.

21. Cotton seeds as claimed in Claim 19 wherein the promoter sequence is selected from the group consisting of the nopaline synthase promoter from Agrobacterium tumefaciens and the cauliflower mosaic virus 35s promoter.

22. A method for introducing genes into cotton plants and plant lines, comprising the following steps in sequence:

a) surface sterilizing cotton seeds;

b) allowing said cotton seeds to germinate thus forming immature cotton plants, said immature cotton plants including hypocotyl tissue;

c) exposing said hypocotyl tissue to a culture of transformation competent non-oncogenic Agrobacterium tumefaciens harboring a Ti plasmid having a T-DNA region including both a foreign chimeric gene and a selection agent resistance gene;

d) culturing said hypocotyl tissue on a medium containing at least one antibiotic toxic to said Agrobacterium tumefaciens but not toxic to cotton cells;

e) culturing said tissue of step d) in the presence of a selection agent for which the resistance gene encodes for resistance so as to select for plant cells transformed with the T-DNA region;

f) inducing somatic embryo formation in the exposed tissue in culture; and

g) regenerating the somatic embryos into whole cotton plants.

23. A cotton plant comprising in the genome of at least some of its cells a foreign gene construction including promoter and control sequences effective in cotton cells and a heterologous coding sequence, the foreign gene construction effective to cause expression of a detectable cellular product coded by the heterologous coding sequence in the plant cells, the cellular product selected from the group consisting of a foreign protein and a negative strand RNA.

2. A method of introducing genes into cotton plants and plant lines comprising the steps of:

exposing hypocotyl tissue of immature cotton plants to a culture of transformation competent non-oncogenic Agrobacterium tumefaciens harboring a Ti plasmid having a T-DNA region including both a foreign chimeric gene and a selection agent resistance gene, both genes including appropriate regulatory sequences so as to be expressed in the cells of cotton plants;

culturing the exposed tissue in the presence of a selection agent for which the resistance gene encodes for resistance so as to select for plant cells transformed with the T-DNA region;

inducing somatic embryo formation in the exposed tissue in culture; and

regenerating the somatic embryos into whole cotton plants.

3. The method of Claim 2 wherein said exposing step is preceded by surface sterilization of cotton seeds followed by germination of said cotton seeds to form said immature cotton plants.

4. The method of Claim 2 wherein the hypocotyl tissue comprises pieces of hypocotyl explants which are removed from said immature cotton plants.

5. The method of Claim 2 wherein the culture of Agrobacterium tumefaciens harbors a binary Ti plasmid system in

which a virulence trait is carried on a plasmid separate from the plasmid carrying the T-DNA region.

6. The method of Claim 5 wherein the T-DNA region includes only the T-DNA right and left borders from the T-DNA of a wild-type Ti plasmid.

7. The method of Claim 2 wherein the selection agent is an antibiotic and the resistance gene codes for antibiotic resistance.

8. The method of Claim 7 wherein the antibiotic resistance gene is the NPTII gene and the antibiotic is selected from the group consisting of Kanamycin and G418.

9. The method of Claim 7 wherein the antibiotic resistance gene is the CAT gene and the antibiotic is Chloramphenicol.

10. The method of Claim 7 wherein two antibiotics and two antibiotic resistance genes are used.

11. The method of Claim 10 wherein in the two antibiotics, one is selected from the group consisting of Hygromycin B and Chloramphenicol, and one is selected from the group consisting of Kanamycin and G418.

12. The method of Claim 2 further including, after the step of exposing the tissue to said Agrobacterium tumefaciens, culturing the tissue on a medium containing at least one antibiotic toxic to said Agrobacterium tumefaciens but not toxic to cotton cells.

13. The method of Claim 2 wherein the step of inducing embryo formation includes culturing the plant tissue on a culture media containing at least one auxin or cytokinin.

14. Cotton plants produced by the method of Claim 2 which include cells which comprise in their genome the foreign chimeric recombinant gene and the selection agent gene and which produce a foreign cellular product coded by the foreign gene.

15. Cotton somatic embryos produced by the method of Claim 2.

16. Cotton seeds produced by the plants of Claim 14.

17. Cotton seed capable of germination into cotton plants comprising in their genome a chimeric recombinant gene construction including a foreign gene and promoter and control sequences operable in cotton cells, the chimeric gene construction being effective in the cells of the cotton plant to express a cellular product coded by the foreign gene, the cellular product imbuing the plant with a detectable trait

selected from the group consisting of a foreign protein and a negative strand RNA.

18. Cotton plants germinated from the seeds of Claim 17.

19. Cotton seeds as claimed in Claim 17 wherein the cellular product is selected from the group consisting of an exogenous protein and an RNA selected to produce a somatic change to the cotton plant.

20. Cotton seeds as claimed in Claim 17 wherein the foreign gene codes for the production of a negative RNA strand effective to condition a somatic change in the cotton plant grown from the seed.

21. Cotton seeds as claimed in Claim 19 wherein the promoter sequence is selected from the group consisting of the nopaline synthase promoter from Agrobacterium tumefaciens and the cauliflower mosaic virus 35s promoter.

22. A method for introducing genes into cotton plants and plant lines, comprising the following steps in sequence:

a) surface sterilizing cotton seeds;

b) allowing said cotton seeds to germinate thus forming immature cotton plants, said immature cotton plants including hypocotyl tissue;

c) exposing said hypocotyl tissue to a culture of transformation competent non-oncogenic Agrobacterium tumefaciens harboring a Ti plasmid having a T-DNA region including both a foreign chimeric gene and a selection agent resistance gene;

d) culturing said hypocotyl tissue on a medium containing at least one antibiotic toxic to said Agrobacterium tumefaciens but not toxic to cotton cells;

e) culturing said tissue of step d) in the presence of a selection agent for which the resistance gene encodes for resistance so as to select for plant cells transformed with the T-DNA region;

f) inducing somatic embryo formation in the exposed tissue in culture; and

g) regenerating the somatic embryos into whole cotton plants.

23. A cotton plant comprising in the genome of at least some of its cells a foreign gene construction including promoter and control sequences effective in cotton cells and a heterologous coding sequence, the foreign gene construction effective to cause expression of a detectable cellular product coded by the heterologous coding sequence in the plant cells, the cellular product selected from the group consisting of a foreign protein and a negative strand RNA.

NJS6/08